

PATENT COOPERATION TREATY

REC'D 08 JUL 2005

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WIPO

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/DK2005/000236

International filing date (day/month/year)
07.04.2005

Priority date (day/month/year)
07.04.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/75, C12N1/21

Applicant
NOVOZYMES AS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☐ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



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Fax: +31 70 340 - 3016

Authorized Officer

Montero Lopez, B

Telephone No. +31 70 340-3739



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/DK2005/000236

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
☒ a sequence listing
☐ table(s) related to the sequence listing
 - b. format of material:
☒ in written format
☒ in computer readable form
 - c. time of filing/furnishing:
☒ contained in the international application as filed.
☒ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

Reference is made to the following document:

D1: KYOKO KANAMARU ET AL.: "Overexpression of the PepF oligopeptidase inhibits sporulation initiation in *Bacillus subtilis*" JOURNAL OF BACTERIOLOGY, vol. 184, no. 1, January 2002 (2002-01), pages 43-50, XP002334436

1. The present application relates to the production of heterologous proteins in *Bacillus* cells which have been mutated to reduce the expression-levels of the YusZ or YusX genes.
2. Document D1 discloses that *B. subtilis* YusX gene encode an oligoendopeptidase and that YusX and YusY may be a single ORF. The function of the YusXY protein appears to be unknown. There is no hint in the state of the art to indicate the skilled person that reduced expression of YusX or YusZ in *Bacillus* would improve secretion of heterologous proteins. Claims 1-57 therefore are novel and inventive and comply with the requirements of Articles 33(2) and (3) PCT.

Re Item VIII

Certain observations on the international application

1. Claims 1, 5, 6, 20, 25, 39 and 44 include the sequence of the Yus protein or polynucleotide between parenthesis. This renders the sequence an optional feature which does not have any limiting effect in the scope of the claims. The deletion of the parenthesis and specification of the sequence would improve the clarity of the claims as requested according to Article 6 PCT.
2. The relative term "homologue" used in claims 1, 5-8, 10-13, 20, 24-27, 29-32, 39, 43-46, 48, 50 and 51 has no well-recognised meaning and leaves the reader in doubt as to the

meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT). It is unclear what sort of homology (functional, structural) is meant and what the degree of homology should be.

3. The relative term "polypeptide of interest" used in claims 1, 20 and 39 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

4. The feature of claims 6-11, 25-30 and 44-49 of a mutation in the YusY gene is not supported by the examples. The examples show only the expected effect for YusX and YusZ deletion mutants. Claims 6-11, 25-30 and 44-49 are therefore not supported by the description as required by Article 6 PCT.

5. Claims 19, 38 and 57 include an optional feature (preferably derived from the CryIIIa promoter) which does not have any limiting effect in the scope of the claims. The deletion of this feature would improve the clarity of the claims as requested according to Article 6 PCT.

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